American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)

ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

Evaluation of Antibacterial Activity of Various Commercial Essential Oils

Sabrine El Adab[a]*, Lobna Mejri[b], Imen Zaghbib[c], Mnasser Hassouna[d]

[a,b,c,d]*Research Unity "Bio-Preservation and Valorization of Agricultural Products–UR 13 AGR02", High Food Industries School of Tunisia, 58 Avenue Alain Savary, Tunis El Khadra 1003, Tunisia*

[a]*Email: eladabsabrina@gmail.com*

[b]*Email: lobnamassoud@gmail.com*

[c]*Email: zaghbibimen@gmail.com*

[d]*Email: Mnasser.Hassouna@isbb.rnu.tn*

Abstract

Essential oils and their components are becoming increasingly popular as naturally occurring antimicrobial agents. In this work the chemical composition and the antibacterial properties of the essential oils of rosemary (*Rosmarinus officinalis*), lemon (*Citrus limonum*), oregano (*Corydothymus capitatus*) and thyme (*Thymus vulgaris*) were determined. The essential oil components were identified by GC/MS analysis. The antibacterial activity of the oils was investigated in order to evaluate its efficacy against a panel of standard reference strains, using disc diffusion and minimum inhibitory concentration (MIC) methods. The GC/MS analysis showed that the major constituents of the oils were monoterpene hydrocarbons and phenolic monoterpenes, but the concentration of these compounds varied greatly among the oils examined. The results of the antibacterial assay showed that *Corydothymus capitatus* and *Thymus vulgaris* have the strongest antibacterial activity against all microorganisms tested. The MIC values obtained in the presence of *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella arizonae* were ≤ 0.25% (v/v) for oregano and thyme essential oils.

Keywords: Antibacterial activity; Citrus limonum; Corydothymus capitatus; MIC; Thymus vulgaris.

---

* Corresponding author.

1. Introduction

Foodborne illness resulting from consumption of food contaminated with pathogenic bacteria has been of vital concern to public health [1]. Currently there is a strong debate about the safety aspects of chemical preservatives since they are considered responsible for many carcinogenic attributes as well as residual toxicity [2]. For these reasons, the use of natural products as antibacterial compounds for food preservation receive increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives [3]. In fact these natural products seem to be an interesting way to control the presence of pathogenic bacteria and to extend the shelf life of processed food. Among these products, essential oils (EOs) from spices, medicinal plants and herbs have been shown to possess antimicrobial activities and could serve as a source of antimicrobial agents against food pathogens [4,5]. Essential oils and their compounds are known to be active against a wide variety of microorganisms, including Gram-negative [6,3] and Gram-positive [5]. Gram-negative bacteria were shown to be generally more resistant than Gram-positive ones to the antagonistic effects of essential oils because of lipopolysaccharide present in the outer membrane [7] but this was not always true [8]. The antimicrobial activity of essential oils is assigned to a number of small terpenoids and phenolic compounds (thymol, carvacrol, eugenol), which also in pure form demonstrate a high antibacterial activity [9].

The aim of the present work was to evaluate antibacterial activity parameters of some essential oils as rosemary (*Rosmarinus officinalis*), lemon (*Citrus limonum*), oregano (*Corydothymus capitatus*) and thyme (*Thymus vulgaris*).

2. Materials and Methods

2.1 Microbial strains

The antibacterial activity was evaluated against a panel of microorganisms, including: (i) five pathogenic microorganisms viz. three Gram-negative: *Pseudomonas aeruginosa* ATCC9027, *Salmonella arizonae* ATCC25922, *Escherichia coli* DH5a and two Gram-positive: *Staphylococcus aureus* ATCC25923, *Listeria monocytogenes* TCC070101121 (Pasteur Institut, Tunisia); (ii) two psychrotrophic bacteria: *Pseudomonas fluorescens* DMS 50090, *Aeromonas hydrophila* ATCC7966 (Research Unity "Bio-Preservation and Valorization of Agricultural Products", Tunisia). Before they were used, the pathogens were cultured in Muller Hinton broth (MHB) (Biokar, Beauvais, France) for 24 h at 37°C and for 24 h at 30°C for psychrotrophic bacteria.

2.2 Essential oils

The essential oils used in this work were purchased from pharmacy Makni (Manouba, Tunisia), which supplies food grade oils. The oils used were rosemary (*Rosmarinus officinalis*), lemon (*Citrus limonum*), oregano (*Corydothymus capitatus*) and thyme (*Thymus vulgaris*). All samples were stored at 4 °C before use.

2.3 Gas chromatography-mass spectrometry (GC-MS) analysis

Volatile compounds analysis by GC-MS was performed on an Agilent 7890A GC system, coupled to an Agilent 5972C mass spectroscopy detector with electron impact ionization (70 eV). A HP-5 MS capillary column (30 m x 0.25 mm, coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 µm film thickness; Hewlett-Packard, CA, USA) was used. The column temperature was programmed to rise from 40 to 240°C at a rate of 5°C min^{-1}.

The carrier gas was helium N60 with a flow rate of 0.9 mL min^{-1}; split ratio was 100:1. Scan time and mass range were 1s and 50-550 m z^{-1}, respectively. The identification of the compounds was based on mass spectra (compared with Wiley Registry 9th Edition/NIST 2011 edition mass spectral library).

2.4 Determination of sensitivity

Disc diffusion method was used for the evaluation of antibacterial activity of essential oils using 100 µL of suspension containing 10^8 CFU mL^{-1} of bacteria spread on Mueller Hinton Agar (MHA) (Biolife, Milan, Italy) [10].

The filter paper dishes (6 mm in diameter) were impregnated with 10 µL and 20 µL of the oil and then placed onto agar diffusion. Thereafter, the plates were kept at 4 °C for 1 h and then incubated at 37 °C for 24 h for bacteria and at 30 °C for 24 h for psychrotrophic bacteria.

Antibacterial activity was evaluated by measuring the zone of inhibition in mm against the test organisms. The sensitivity to the different oils was classified by the diameter of the inhibition halos as follows: not sensitive (-) for diameter less than 8 mm; sensitive (+) for diameter 9-14 mm; very sensitive (++) for diameter 15-19 mm and extremely sensitive (+++) for diameter larger than 20 mm [11].

The experiments were repeated in triplicate and the results were expressed as average values.

2.5 Determination of minimum inhibitory concentration (MIC)

The MIC of the tested essential oils was determined using a broth dilution method (Joshi and his colleagues 2010; Jrah Harzallah and his colleagues 2011). The MIC is defined as the minimum level of essential oil concentration that produces a 90% reduction in the growth (populations) of microbial colonies [12].

All tested were perfomed in Muller Hinton broth (Biokar) supplemented with tween 80 (final concentration of 0.5%, v/v) to enhance the oil solubility. Different concentrations of the essential oils tested (0- 0.03- 0.06- 0.12- 0.25- 1- 2- 4%, v/v) were prepared in broth. The essential oils used in this study were the oils with the highest antibacterial activity.

Salm. arizonae ATCC25922, *E. coli* DH5a, *Staph. aureus* ATCC25923 and *L. monocytogenes* TCC070101121 were enriched in Mullen Hinton broth (Biokar) and incubated for 24 h at 37 °C. A volume of 100 µL of the inoculums containing approximately 10^7-10^8 CFU mL^{-1} microorganisms was added to the different solutions of oils. All the tubes were then incubated at 37 °C for 24 h. The determination of MIC is based on the

measurement of the cell concentration (CFU mL^{-1}).

2.5 Statistical analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA) procedure of SPSS 17.0 (SPSS, Inc., Chicago, IL). Duncan's multiple range test was used to determine any significant difference between mean values and evaluations were based on a significance level of $P < 0.05$.

3. Results and Discussion

3.1 Chemical composition of the essential oils

The major components in oregano, thyme, rosemary and lemon EOs are essentially monoterpenes phenolics and monoterpene hydrocarbons (Table 1). The main components of rosemary essential oil were 1,8-cineole (43.57%) and camphor (15.26%). Our results are in agreement with those obtained in [13].

The EO of lemon is reaches in monoterpenes with the majority compound limonene, whose content reaches 56.10%. The authors in [9] showed that the major component of the EO of citrus is limonene, which varies from 45 to 76% for lemon EO. β-Linalool (79.17%), thymol (6.58%) and caryophyllene (6.11%) were the most representative components of thyme EO. Some variances between our results and others were seen, as the authors in [14] reported 21.89% and 3.63% for carvacrol and thymol, respectively.

According to the authors in [14], these variances were due to the difference in herbal species, their ecotypes and other environmental parameters. At least, seven chemotypes of *Thymus vulgaris* exist.

Carvacrol (74.87%), thymol (2.54%), o-cymene (9.74%), δ-terpinene (4.16%) and caryophyllene (2.07%) were the principal constituents of oregano EO. Our results are in agreement with other study which reported that carvacrol (76%) followed by thymol (5%) were the major components of *corydothymus capitatus* [1].

3.2 Antibacterial activity

Antibacterial inhibition zones of essential oils against seven pathogenic bacteria were showed in Table 2.

With inhibition zones ranging from 9 to 20 mm, all pathogenic bacteria were classified as susceptible strains to rosemary essential oil. *Staph. aureus*, *Salm. arizonae* and *Aer. hydrophila* showed high susceptibility to rosemary EO; with inhibition zones ranging from 14 to 20 mm. The authors in [15] found that rosemay EO had a strong antibacterial activity especially against *Staph. aureus* and *E. coli*.

In fact, rosemary EO is mainly composed of 1,8 cineole (43.57%), camphor (15.26%). These compounds increase the antibacterial activity of terpenoids. In addition, minor compounds may participate to the antibacterial activity of the oil [9].

D: essential oil dose tested (µL).

Ps. aeruginosa, *Salm. arizonae*, *Ps. fluorescens* and *Aer. hydrophila* were not sensitive to lemon EO. However *Staph. aureus*, *L. monocytogenes* and *E. coli* were sensitive to lemon EO and this for a dose of 20 µL. our results are in agreement with those obtained in [16] who reported that *E. coli* was sensitive to lemon EO, showing inhibition zones ranging from 9 to 11 mm.

Table 1: Chemical composition of oregano, thyme, lemon and rosemary essential oils

compound	**Area (%)**			
	Corydothymus capitatus	*Thymus vulgaris*	*Citrus limonum*	*Rosmarinus officinalis*
	Spanish oregano	Linalol thyme	Lemon	Cineole rosemary
	Flowering plant	Flowering plant	Fruit peel	Leaf
α-Thujene	0.834	0.17	0.60	0.39
α-Pinene	0.802	0.13	8.64	12.00
Camphene	0.073	0.13	0.16	4.75
Hydroxy-1-octene	0.043	0.18	n.d	n.d
β-Pinene	0.073	0.06	11.54	5.83
β-Myrcene	0.735	0.17	1.29	1.08
l-Phellandrene	0.108	n.d	0.04	0.17
Delta-3-Carene	0.047	n.d	2.94	0.04
α-Terpinene	1.044	0.09	0.12	0.42
o-Cymene	9.974	n.d	1.99	2.25
dl-Limonene	0.136	0.12	n.d	2.23
δ -Terpinene	4.161	0.91	8.84	0.59
Cis-Sabinene hydrate	0.121	n.d	n.d	n.d
α-Terpinolene	0.087	n.d	0.70	0.32
Linalool	0.808	n.d	n.d	n.d
Borneol	0.061	n.d	n.d	n.d
Terpinene-4-ol	0.688	n.d	n.d	0.69
α-Terpineol	0.061	0.31	n.d	1.59
Thymol	2.540	6.58	n.d	n.d
Carvacrol	74.866	n.d	n.d	n.d
o-Thymol	0.079	n.d	n.d	n.d
Caryophyllene	2.074	6.11	0.24	4.19
α-Humulene	0.052	0.15	0.02	0.35
Caryophyllene oxide	0.533	n.d	n.d	n.d
3-Octanol	n.d	n.d	n.d	n.d
p-Cymene	n.d	n.d	n.d	n.d
1,8-Cineole	n.d	0.19	n.d	43.57

Table 1: Continued

compound	Area (%)			
	Corydothymus capitatus	*Thymus vulgaris*	*Citrus limonum*	*Rosmarinus officinalis*
	Spanish oregano	Linalol thyme	Lemon	Cineole rosemary
	Flowering plant	Flowering plant	Fruit peel	Leaf
Trans Linalool oxide	n.d	0.04	n.d	n.d
Cis Linalool oxide	n.d	0.07	n.d	n.d
β-Linalool	n.d	79.17	n.d	0.68
Camphor	n.d	0.13	n.d	15.26
1-Borneol	n.d	0.11	n.d	n.d
4-Terpineol	n.d	0.08	0.18	n.d
Thymol methyl ether	n.d	0.05	n.d	n.d
Linalyl acetate	n.d	2.35	n.d	n.d
Bornyl acetate	n.d	0.03	n.d	0.33
Linalol	n.d	n.d	0.56	n.d
Cis-Limonene oxide	n.d	n.d	0.08	n.d
Trans-Limonene oxide	n.d	n.d	0.07	n.d
4,8-Epoxy-p- menth-1-ene	n.d	n.d	0.09	n.d
β-Fenchyl alcohol	n.d	n.d	0.15	n.d
2,3-epoxygeranial	n.d	n.d	0.08	n.d
Z-Citral	n.d	n.d	0.43	n.d
E-Citral	n.d	n.d	0.70	n.d
p-Menth-1-ene, 8,9-epoxy	n.d	n.d	0.07	n.d
Camphene	n.d	n.d	0.26	n.d
Nerol acetate	n.d	n.d	0.36	n.d
Nerol	n.d	n.d	0.20	n.d
α-Copaene	n.d	n.d	0.06	n.d
Trans-α-Bergamotene	n.d	n.d	0.42	n.d
δ -Murolene	n.d	n.d	0.02	n.d
Cis-α-Bisabolene	n.d	n.d	0.05	n.d
β-Bisabolene	n.d	n.d	0.53	0.04
Delta-Cadinene	n.d	n.d	0.05	n.d
Spathulenol	n.d	n.d	0.01	n.d
Cedrol	n.d	n.d	0.25	n.d
α-Myrcene	n.d	n.d	n.d	0.14
D-Fenchyl alcohol	n.d	n.d	n.d	0.03

Isopulegol	n.d	n.d	n.d	0.04
Borneol	n.d	n.d	n.d	2.67
2-Methyl-1-nonene-3-yne	n.d	n.d	n.d	0.03
l-Verbenon	n.d	n.d	n.d	0.06
α-Ylangene	n.d	n.d	n.d	0.03
α-Cubebene	n.d	n.d	n.d	0.04
Trans-Caryophyllene	n.d	n.d	n.d	0.11
Cis-Caryophyllene	n.d	n.d	n.d	0.02
7-epi-α-Cadinene	n.d	n.d	n.d	0.03
Delta-Cadinene	n.d	n.d	n.d	0.04
Total	100	100	100	100

*, according to the data of the gas chromatography analysis of essential oils; n.d, not detected.

Table 2: Zone of inhibition of growth of 7 different microorganisms by essential oils

Microorganism	**Inhibition zone diameter (mm)[a]**							
	Rosmarinus officinalis		*Citrus limonum*		*Thymus vulgaris*		*Corydothymus capitatus*	
	D_{10}	D_{20}	D_{10}	D_{20}	D_{10}	D_{20}	D_{10}	D_{20}
Staphylococcus aureus ATCC 25923	14 ± 0.05	15 ± 0.33	8 ± 0.4	12,5 ± 0.02	19 ± 0.03	31 ± 1.22	38 ± 0.01	47 ± 0.06
Listeria monocytogenes TCC 070101121	11 ± 0.03	13 ± 0.02	8 ± 0.03	10 ± 0.02	20 ± 0.5	25 ± 0.02	31 ± 0.00	44 ± 0.03
Escherichia coli DH5α	9 ± 0.22	13 ± 0.5	9 ± 0. 01	11 ± 0.2	18 ± 0.00	23 ± 0.02	33 ± 1.21	37 ± 0.01
Pseudomonas aeruginosa ATCC 9027	9 ± 0.03	10 ± 0.02	6,5 ± 0.01	8 ± 0.01	11 ± 0.00	14 ± 0.01	33 ± 0.02	41 ± 0.00
Salmonella arizonae ATCC 25922	18,5 ± 0.01	20 ± 0.01	7 ± 0.03	7 ± 0.01	19 ± 0.02	23 ± 0.3	31 ± 0.01	41 ± 0.00
Aeromonas hydrophila ATCC 7966	14 ± 0.22	20 ± 0.04	6,5 ± 0.01	7 ± 0.34	14 ± 0.01	19 ± 0.01	33 ± 0.00	39 ± 0.02
Pseudomonas fluorescens DMS 50090	8 ± 0.01	11 ± 0.01	7 ± 0.00	7 ± 0.01	13 ± 0.01	16 ± 0.04	31 ± 0.01	43 ± 0.01

[a] The diameter of the filter paper discs (6 mm) is included.

The author in [17] reported that *Staph. aureus* had a variable sensitivity to essential oils of some citrus species tested. In fact, essential oils of lemon and bergamot showed respectively zones of inhibition of 30 mm and 12 mm against *Staph. aureus*. The sensitivity of this microorganism to lemon EO was also observed by the authors

in [18,9].

The authors in [19] showed that lemongrass EO has an antimicrobial activity against Gram-positive bacteria such as *Staph. aureus* and *L. monocytogenes* but did not show any effect on the Gram-negative such as *Salm. thyphimurium* and *E. coli*.

Our results showed that thyme EO had a strong antibacterial activity against all pathogenic bacteria, with inhibition zones ranging from 11 to 31 mm. In fact, the most sensitive bacteria to thyme EO were *Staph. aureus* followed by *E. coli* and *Salm. arizonae*. Our results are in agreement with many other studies [20,21] reporting that thyme EO had a strong antibacterial activity against pathogenic bacteria.

Ps. aeruginosa and *Ps. fluorescens* showed low susceptibility to thyme EO. Our results are in agreement with those obtained in [22] who reported that thyme EO had a strong antibacterial activity against the different strains with the exception of *Ps. aeruginosa* and *Ps. fluorescens*. In fact, *Ps. aeruginosa* is resistant to many antiseptics and disinfectants since it possesses a higher level of Mg^{2+} in its outer membrane. Higher level of magnesium in the membrane will increase the cross linking between the LPS therefore, reduce the size of the porine and ultimately limit the migration of antimicrobials molecules through the bacterial membrane [23].

Oregano EO had a strong antibacterial activity against all pathogenic bacteria. In fact, the most sensitive bacteria to oregano EO was *Staph. aureus*, with inhibition zones ranging from 31 to 47 mm, followed by *L. monocytogenes*, with inhibition zones ranging from 31 to 44 mm. our results are in agreement with many other studies [24,25,26] reporting that oregano EO had a strong antibacterial activity.

The authors in [27] reported the powerful inhibitory effect of oregano EO on the growth of *E. coli* O157: H7, *L. monocytogenes*, *Salm. thyphimurium* and *Staph. aureus*. The antibacterial efficiency of oregano EO can be related to the concentration and proportion of phenolic compounds [28]. Carvacrol, thymol, γ-therpinene and o-cymene were the most active constituents of oregano EO, with a wide spectrum of antimicrobial property [29,9]. The author in [9] described the action of carvacrol as the disintegration of the external membrane of Gram-negative bacteria followed by the release of the lipopolysaccharides present, resulting in increased permeability of the cytoplasm membrane to ATP.

The authors in [5] observed that carvacrol was the most active constituent of 11 EOs tested and this constituent present at high concentration in *Corydothymus capitatus* and *Thymus vulgaris*. The authors in [30] showed that *B. cereus*, *E. coli*, *L. monocytogenes* and *Ps. aeruginosa* were sensitive to oregano EO. There seemed to be no difference in antibacterial effects between Gram-positive and Gram-negative bacteria.

On the basis of these results, it is possible to conclude that the EOs of oregano and thyme have the strongest antibacterial activity between all essential oils tested.

According to the potential of antibacterial activities of EOs, we can classify the EOs as follows: lemon < rosemary < thyme < oregano.

3.3. Minimum inhibitory concentration determination

The MIC values obtained in the presence of *E. coli*, *L. monocytogenes*, *Staph. aureus* and *Salm. arizonae* were ≤ 0.25% (v/v) for oregano and thyme EOs (Table 3). Our results showed that oregano and thyme EOs exhibited an antibacterial effect against the four pathogenic bacteria, without any significant difference ($p > 0.05$) between them.

Table 3: Minimum inhibitory concentrations (MICs) of selected essential oils (% v/v) against 4 different microorganisms

Essential oil	**Microorganism**			
	Echerichia coli DH5α	*Listeria monocytogenes* TCC 070101121	*Staphylococcus aureus* ATCC 25923	*Salmonella arizonae* ATCC 25922
Corydothymus capitatus	0.06	0.03	0.12	0.25
Thymus vulgaris	0.25	0.03	0.12	0.25

The MIC of oregano EO against *E. coli* was 0.06% (v/v). Our results are in agreement with those obtained by [28] Hammer and his colleagues (1999). In addition, oregano EO exhibited an antibacterial effect against *L. monocytogenes* with a MIC of 0.03%. The same result was obtained in [30]. Furthermore, our results are not in agreement with those obtained by the authors in [1] who found that *Staph. aureus* and *Salm. arizonae* showed MICs < 0.05% (v/v).

Regarding the thyme EO, the MIC against *E. coli* was 0.25% which is not in agreement with the results obtained by the authors in [31] who showed a MIC of 0.12% against *E. coli*.

The MIC of thyme EO against *L. monocytogenes* was 0.03% which is in agreement with the results obtained by the authors in [32] who reported that MIC of thyme EO against *L. monocytogenes* was 0.02%.

The composition and antibacterial activity of EOs were very different. A similar trend was observed by the authors in [33] which showed that there were considerable variations between the antibacterial actions of essential oils. However, the comparison of the efficiency of the oils between studies is difficult due to different uncontrollable and external parameters. The composition of plant oils is known to vary according to local climatic and environmental conditions. Moreover, the antimicrobial properties can vary within the same plant species because the chemical composition and relative proportions of the individual constituents in the essential oils of the plant are influenced by genotype [34]. Besides, some oils with the same common name may be derived from different plant species. Similarly, the method used to assess antimicrobial activity, and the choice of tested microorganisms, various between publications. Agar and broth dilution methods are also commonly used. The results obtained by each of these methods may differ as many factors vary between assays [35]. These

include differences in microbial growth, time of exposition of microorganisms to plant oil, the solubility of oil and the method to solubilize or emulsifying them. These and other elements may account for the large differences in MICs obtained by the broth dilution method in this study.

4. Conclusion

Food contamination is still an enormous public health problem, but may be better controlled by the use of natural preservatives. Among the essential oils tested in this study, *Corydothymus capitatus* and *Thymus vulgaris* showed the strongest antibacterial activity. As food preservatives, volatile oils may have their greatest potential use. Although, even if numerous essential oils possess antimicrobial properties, their strong flavoring properties will ultimately limit their usage as food antimicrobial agents. However, a study had been undertaken in a Tunisian dry fermented poultry meat sausage to confirm the antimicrobial efficiency level of these essential oils, and their organoleptic impact [3].

5. Recommendations

The essential oils and their main active components could be potential candidates to be used as natural alternatives for further application in food preservation to inhibit the bacterial growth and to extend the shelf life of the food products. However, the confirmation of antimicrobial efficiency and organoleptic impact of these essential oils in foodstuffs need to be evaluated.

Acknowledgments

The authors thank the Center of Biotechnology of Borj-Cedria (CBBC) (Borj-cedria, Ben Arous, Tunisia) for providing part of the equipment used in this work.

References

[1] M. Oussalah, S. Caillet, L. Saucier and M. Lacroix, “Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157:H7, Salmonella Typhimurium, Staphylococcus aureus and Listeria monocytogenes,” Food Control, vol. 18, pp. 414-420, 2007.

[2] P. Skandamis, K. Koutsoumanis, K. Fasseas and G. J. E. Nychas, “Inhibition of oregano essential oil and EDTA on E. coli O157:H7,” Italian Journal of Food Science, vol. 13, pp. 55-65, 2001.

[3] S. El Adab, and M. Hassouna, “Proteolysis, lipolysis and sensory characteristics of a Tunisian dry fermented poultry meat sausage with oregano and thyme essential oils,” Journal of Food Safety, vol. 36, pp. 19-32, 2016.

[4] S. G. Deans and G, Ritchie, “Antibacterial properties of plant essential oils,” International Journal of Food Microbiology, vol. 5, pp. 165-180, 1987.

[5] J. Kim, M. R. Marshall and C. I. Wei, “Antibacterial activities of some essential oil components against

five foodborne pathogens," Journal of Agricultural and Food chemistry, vol. 43, pp. 2839-2845, 1995.

[6] I. M. Helander, H. L. Alakomi, K. Latva-Kala, T. Mattila-Sandholm, I. Pol, E. J. Smid, L.G.M. Gorris and A. Von Wright, "Characterization of the action of selected essential oil components on Gram-negative bacteria," Journal of Agricultural and Food chemistry, vol. 46, pp. 3590-3595, 1998.

[7] A. D. Russel, "Mechanisms of bacterial resistance to non-antibiotics: food additives and food pharmaceutical preservatives," Journal of Food Applied bacteriology, vol, 71, pp. 191-201, 1991.

[8] M. Karapinar and S. E. Askun, "Inhibition of foodborne pathogens by thymol, eugenol, menthol and anethole," International J ournal of Food Microbiology, vol. 4, pp.161-166, 1987.

[9] S. Burt, "Essential oils: their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology," vol. 94, pp. 223-253, 2004.

[10] A. I. Hussain, F. Anwar, S. A. S. Chatha, A. Jabbar, S, Mahboob and P. S. Nigam, "Rosmarinus officinalis essential oil: antiproliferative, antioxidant and antibacterial activities," Brazilian Journal of Microbiology, vol. 41, pp. 1070-1078, 2010.

[11] A. G. Ponce, R. Fritz, C. E. Del Valle and S. I. Roura, "Antimicrobial activity of essential oils on native microbial population of organic Swiss chard," Lebensmittel-Wissenschaft und-Technology, vol. 36, pp. 679-684, 2003.

[12] P, Skandamis, K, Koutsoumanis, K. Fasseas and G. J. E. Nychas, "Inhibition of oregano essential oil and EDTA on E. coli O157:H7," Italian Journal of Food Science, vol. 13, pp. 55-65, 2001.

[13] J. Gutierrez, C. Barry-Ryan and P. Bourke, "Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components," Food Microbiology, vol. 26, pp. 142-150, 2009.

[14] V. Chamanara, B. Shabanpour, S. Gorgin and M. Khomeiri, "An investigation on characteristics of rainbow trout coated using chitosan assisted with thyme essential oil," International Journal of Biological Macromoecules, vol. 50, pp. 540-544, 2012.

[15] S. Ayadi, C. Jerribi and M. Abderraba, "Extraction and study of essential oils of Rosmarinus officinalis harvested in different regions of Tunisia," Journal of Algebra Chemical Society, vol. 21, pp. 25-33, 2011.

[16] M. R. Moreira, A. G. Ponce, C. E. Del Valle and S. I. Roura, "Inhibitory parameters of essential oils to reduce a foodborne pathogen," Food Science Technology, vol. 38, pp. 565-570, 2005.

[17] V. G. De Billerbeck, "Essential oils and antibiotic resistant bacteria," Phytotherapy, vol. 5, pp. 29-253, 2007.

[18] S. D. Cox, J. L Mann, H. C. Markham, J. E. Bell and J. R. Gustafson, "The mode of antimicrobial action of the essential oils of Malaleuca alternifolia.," Journal of Applied Microbiology, vol. 88, pp. 170-177, 2000.

[19] P. Kotzekidou, P. Giannakidis and A. Boulamatsis, "Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated pathogens in chocolate," LWT- Food Science Technology, vol. 41, pp. 119-127, 2008.

[20] A. Smith-Palmer, J. Stewart and L. Fyfe, "Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens," Letters in Applied Microbiology, vol. 26, pp. 118-122, 1998.

[21] A. El Oualilalami, F. El-Akhal, W. Ouedrhiri, F. Ouazzani Chahdi, R. Guemmouh and H. Greche, "Thymus essential oils (Thymus vulgaris and Thymus Satureioïdis) from center of Maorocco: chemical composition and antimicrobial activity," Journal of Medical Laboratory Technology, vol. 8, pp. 27-33, 2013.

[22] F. Haddouchi, H. A. Lazouni, A. Meziane and A. Ben Mansour, "Physicochemical and microbiological study of the essential oil of Thymus fontanesii Boiss & Reut," Science in Africa, vol. 5, pp. 246-259, 2009.

[23] G. McDonnell and A. D. Russell, "Antiseptics and disinfectants: activity, action and resistance," Clinical microbiology Reviews, vol. 12, pp. 147, 1999.

[24] E. Chouliara, A. Karatapanis, I. Savvaidis and M. G. Kontominas, "Combined effect of oregano oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C," Food Microbiology, vol. 24, pp. 607-617, 2007.

[25] T. Askun, G. Tumen, F. Satil and M. Ates, "Characterization of the phenolic composition and antimicrobial activities of Turkish medicinal plants," Pharmaceutical Biology, vol. 47, pp. 563-571, 2009.

[26] A. A. Dobre, V. Gagiu and N. Petru, "Antimicrobial activity of essential oils against food-borne bacteria evaluated by two preliminary methods," Romanian Biotechnological Letters, vol. 16, pp. 119-125, 2011.

[27] I. Dadalioglu and G. Evrendilek, "Chemical composition and antibacterial effects of essential oils of Turkish oregano (Origanum minutuflorum), baylaurel (Laurusnobilis), Spanish lavender (Lavandulastoechas L.) and fennel (Foeniculum vulgare) on common foodborne pathogens," Journal of Agricultural Food Chemistry, vol. 52, pp. 8255-8260, 2004.

[28] S. Zivanovic, S. Chi and A. F. Draughon, "Antimicrobial activity of chitosan films enriched with

essential oils," Journal of Food Science, vol. 70, pp. 45-52, 2005.

[29] R. J. W. Lambert, P. N. Skandamis, P. Coote and G. J. E Nychas, "A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol," Journal of Applied Microbiology, vol. 91, pp. 453-462, 2001.

[30] J. Gutierrez, C. Barry-Ryan and P, Bourke, P. "Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components," Food Microbiology, vol. 26, pp. 142-150, 2009.

[31] K. A. Hammer, C. F. Carson and T. V. Riley, "Antimicrobial activity of essential oils and other plant extracts," Journal of Applied Microbiology, vol. 86, pp. 985-990, 1999.

[32] A. Smith-Palmer, J. Stewart and L. Fyfe, "Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens," Letters in Applied Microbiology, vol. 26, pp. 118-122, 1998.

[33] M. Lis-Balchin and S. G. Deans, "Bioactivity of selected plant essential oil against Listeria monocytogenes.,"J ournal of Applied Microbiology, vol. 82, pp. 759-762, 1997.

[34] M. L Arrebola, M. C. Navarro, J. Jime´nez and F. A. Oca´na, "Yield and composition of the essential oil of Thymus serpylloides subsp serpylloides," Phytochemistry, vol. 1, pp. 67-72, 1994.

[35] A. M. Janssen, J. J. C. Scheffer and A. Baerheim Svendsen, A. "Antimicrobial activity of essential oils: a literature review. Aspects of the test methods," Planta Medica, vol. 53, pp. 395-398, 1987.